Analysis of ATP2C1 Gene Mutation in 10 Unrelated Japanese Families with Hailey–Hailey Disease

To the Editor:

Hailey–Hailey disease (HHD) is an autosomal dominant chronic blistering disease, which is histologically characterized by keratinocyte acantholysis and epidermal cleft formation (Burge, 1992). The disease typically presents in middle age as crusted erosions or circinate plaques in sites exposed to friction such as the neck, axillae, groin, and perineum. Recently, the gene ATP2C1 has been identified as defective in HHD. It has been suggested that HHD is caused by a haploinsufficiency of this new Ca^{2+}-ATPase (Hu *et al*, 2000; Sudbrak *et al*, 2000). So far 33 different mutations in the ATP2C1 gene from 35 unrelated HHD families have been identified in the literature. Mutations in 42 cases have yet to be identified out of a total of 77 so far analyzed. In this study, we screened the genomic DNA of the ATP2C1 gene in 10 unrelated Japanese families with HHD, and found five mutations including four novel mutations of ATP2C1.

Ten genetically unrelated Japanese patients with HHD were analyzed in this study (**Table I**). There were nine males and one female. They have had no family history of HHD or other skin diseases.

Genomic DNA was extracted from peripheral blood leukocytes using a standard procedure. Pairs of primers spanning all 28 exons and flanking intronic splice sites of the ATP2C1 gene were used to amplify the genomic DNA. Aliquots of the PCR products were analyzed on 2% agarose gel electrophoresis, and 8 μl of the samples were used for conformation sensitive gel electrophoresis (CSGE) analysis (Ganguly *et al*, 1993). Subsequently, PCR products containing the heteroduplexes were subject to direct sequencing by an ABI automated sequencer (PE Applied Biosystems, Foster City, CA). Alternatively, in samples where heteroduplexes were not detected by CSGE analysis, all the PCR products were subject to direct sequencing.

CSGE analysis of PCR fragments detected three abnormal shifts in electrophoretic mobility. Sequencing of these PCR products revealed three different mutations in three cases (**Table I**). One 457C→T base substitution resulted in a nonsense mutation in exon 7 of case 1 (**Fig 1*A***). This mutation was verified by a DdeI digestion. This was identical to a mutation as reported by Hu *et al* (2000). Also detected was a C490F missense mutation in exon 17 resulting from a 1469G→T substitution in case 2 (**Fig 1*B***). As no enzymatic verification could test this mutation, we performed direct sequencing of exon 17 in 50 normal control subjects to verify this mutation. The mutation 2460delG created a shift in the reading frame and resulted in a downstream PTC at exon 25 in case 10 (**Fig 1*D***). In the other seven cases, we conducted direct sequencing of the entire coding sequence of the ATP2C1 gene, because we could not detect any heteroduplex bands. One L584P missense mutation was found in case 3 (**Fig 1*C***), and was confirmed by Nci I digestion. A splice site mutation occurred in case 10 at the site of a guanine of a conserved GT nucleotide within the donor splice site of intron 12 (**Fig 1*E***). This mutation would alter the correct splicing of exon 12 and result in PTC at eight nucleotides downstream from the donor splice site, although the precise transcription product is unknown as mRNA was unavailable from this case. We were unable to detect any nucleotide changes in five cases despite direct sequencing of the entire genomic coding sequence of the ATP2C1 gene (**Table I**).

We have analyzed ATP2C1 mutations in 10 genetically unrelated Japanese families with HHD showing typical clinical and histopathologic features of this condition. We have found five attributable mutations including four novel mutations comprising two missense mutations (C490F and L584P), one deletion (2460delG), and one splice-site mutation (1259+1g→a). We could not determine any ATP2C1 mutations in five of the 10 HHD families after direct sequencing of the entire coding region of ATP2C1 genomic DNA, including the exon–intron

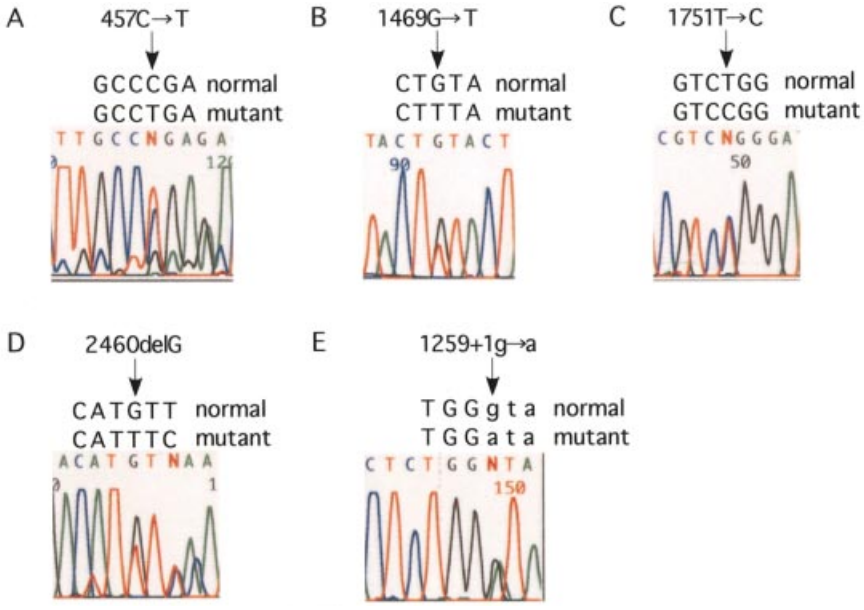

Figure 1. Mutation analysis in HHD families. (*A*) Mutation in case 1. A 457C→T base substitution resulted in a nonsense mutation in exon 7. (*B*) Mutaion in case 2. A 1469G→T substitution resulted in a C490F missense mutation in exon 17. (*C*) Mutation in case 3. A 1751T→C substitution resulted in a L584P missense mutation in exon 19. (*D*) Mutation in case 4. A 2460delG created a shift in the reading frame and resulted in a downstream PTC in exon 25. (*E*) Mutation in case 5. A 1259+1g→a splice site mutation resulted in skipping exon 12.

Manuscript received December 11, 2000; revised October 25, 2001; accepted for publication November 5, 2001.

Reprint requests to: Dr. Koichi Yokota, Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, Japan. Email: koichi@med.hokudai.ac.jp

Table I. ATP2C1 mutations in patients with HHD

Patient	Age/sex	Age of onset	Mutation[a]	Location of mutation	Nucleotide change[b]	Consequence
1	55/M	51	R153X	exon 7	457C→T	nonsense
2	54/M	38	C490F	exon 17	1469G→T	missense
3	70/M	40	L584P	exon 19	1751T→C	missense
4	58/M	40	2460delG	exon 25	CATGTT→CATTT	franeshift (PTC+5 aa)
5	62/F	55	1259+1g→a	intron 12	TGGgta→TGGata	skip exon 12 (PTC)
6	45/M	30	ND			
7	45/M	20	ND			
8	33/M	27	ND			
9	44/M	30	ND			
10	63/M	62	ND			

[a]Numbering of the amino acids refers to the peptide sequence.
[b]Numbering of the nucleotides refers to ATP2C1 cDNA sequence, with the first nucleotide of ATG initiation codon as 1. Bases in the exons are denoted by uppercase letters and bases in the introns by lowercase letters. PTC+n aa indicates that the premature termination codon is ''n'' amino acids downstream of the mutation. X, stop codon; ND, ''not detected''; PTC, premature termination codon.

boundaries. All of the cases analyzed have typical features of HHD suggesting several possible causes of this discrepancy. First, we cannot detect large deletions spanning the entire coding region of ATP2C1 with our detection system. Second, we cannot detect intronic mutations, mutations in the promoter regions, or mutations in the 3′-untranslated region. The fact that Hu *et al* (2000) detected only 21 mutations out of a possible 61 HHD cases using the same primer sets supports our findings.

Among the five families in which we could determine mutations, those with missense mutations (cases 2 and 3) and the one with a nonsense mutation in exon 25 (case 4) were predicted to produce abnormal ATP2C1 protein. These three cases showed early clinical symptoms (before the age of 40) compared with those with nonsense mutations in the 5′ proximal exons (cases 1 and 5). In cases 1 and 5, severely reduced amounts of ATP2C1 protein are expected to be found because of ''nonsense-mediated mRNA decay'' (Frischmeyer and Dietz, 1999; Hentze and Kulozik, 1999). mRNA that has a nonsense mutation at the 5′ proximal region would result in breakage because of the mechanism that is called ''nonsense-mediated mRNA decay'', and no abnormal truncated protein would be translated. On the other hand, mRNA that has a missense mutation or a nonsense mutation close to the end of the gene could be translated, and abnormal protein could interfere the action of normal ATP2C1 protein. Although HHD has been considered to be the result of haploinsufficiency of the ATP2C1 gene, the dominant negative effects of abnormal ATP2C1 protein might also contribute to the disease phenotype. Our data provide a significant addition to the HHD mutation database and will contribute further to the understanding of HHD genotype/phenotype correlations and to the pathogenesis of this disease.

Koichi Yokota, Yasuko Takizawa,* Kana Yasukawa,
Kumiko Kimura, Takeji Nishikawa,* Hiroshi Shimizu
Department of Dermatology,
Hokkaido University Graduate School of Medicine,
Sapporo, Hokkaido, Japan
*Department of Dermatology,
Keio University School of Medicine,
Shinjuku-ku, Tokyo, Japan

REFERENCES

Burge SM: Hailey-Hailey disease: the clinical features, response to treatment and prognosis. *Br J Dermatol* 126:275–282, 1992

Frischmeyer PA, Dietz HC: Nonsense-mediated mRNA decay in health and disease. *Hum Mol Genet* 8:1893–1900, 1999

Ganguly A, Rock MJ, Prockop DJ: Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: evidence for solvent-induced bends in DNA heteroduplexes [published erratum appears in *Proc Natl Acad Sci USA* 91: 5217, 1994]. *Proc Natl Acad Sci USA* 90:10325–10329, 1993

Hentze MW, Kulozik AE: A perfect message: RNA surveillance and nonsense-mediated decay. *Cell* 96:307–310, 1999

Hu Z, Bonifas JM, Beech J, *et al*: Mutations in ATP2C1, encoding a calcium pump, cause Hailey-Hailey disease. *Nat Genet* 24:61–65, 2000

Sudbrak R, Brown J, Dobson-Stone C, *et al*: Hailey-Hailey disease is caused by mutations in ATP2C1 encoding a novel Ca (2+) pump. *Hum Mol Genet* 9:1131–1140, 2000

Unusual Pemphigus Phenotype in the Presence of a Dsg1 and Dsg3 Autoantibody Profile

To the Editor:

Pemphigus foliaceus (PF) and pemphigus vulgaris (PV) are two autoimmune blistering diseases presenting with distinct but related antigenic specificity and histopathologic features (Amagai, 1995; Stanley, 1995; Suter *et al*, 1998; Anhalt and Diaz, 2001). In both diseases, the target antigens are transmembrane components of desmosomal junctions that mediate strong intercellular adhesion between keratinocytes (Green and Gaudry, 2000). In PF, the autoantibodies target desmoglein (Dsg) 1, and in PV Dsg3 (Amagai, 1995; Stanley, 1995; Suter *et al*, 1998; Anhalt *et al*, 2001). Whereas the autoantibodies bind to all sites within stratified squamous epithelia where the antigen is expressed, blister formation is restricted to specific locations. In PF, split formation occurs in the subcorneal zone of the epidermis where Dsg1 is present without Dsg3, and in PV blisters form between basal and suprabasal layers of initially mucous membranes where Dsg3 is present without Dsg1

Manuscript received October 18, 2001; revised November 12, 2001; accepted for publication November 23, 2001.

Reprint requests to: Dr. Eliane Müller, Institute of Animal Pathology, Länggass-Str. 122, 3012 Bern, Switzerland. Email: eliane.mueller@itpa.unibe.ch